Fig. 2. Determination of expression of recombinant ZP3 in transfected PA-1 cells by RT-PCT. (A RT-PCT amplification of first strand of cDNA from the RNA sample of PA-1 cells stable transfected with human ZP3 CDNA with primers A9CH1)/B(CH2) and A/C (B1). Location of PR primers (Primer A5' - TAGGATCCACCATGGACTGAGCTATAGG-3', SEQ ID NO: 1, primer B5'-TTATTCGGAAGCAGACACAGGGTGGGAGGCAGT-3', SEQ ID NO: 2, Primer C 5'-
TTCTCGAGTTAATGATGATGATGATGATGTTCGGAAGCAGACACAGGGTGG
GAGGCAGT-3') SEQ ID NO: 3--

A1

Page 14, second paragraph (lines 13-37), delete the existing paragraph and insert the following new paragraph:

A2

Total RNA was isolated from the human ovary (the utilization of human tissue was approved by the Institutional Review Board of Eastern Virginia Medical School) by using the guandium thiocyanate method (Chirgwin, et al, 1979). A pair of primers was designed based on the published sequence of hZP3 cDNA with additional restriction enzyme sites and histidine tail (Chamberlin and Dean, 1990). The sense primer was located between base 1 to 22 with Bam HI site in the 5' end (5'-TAGGATCCACCATGGAGTGAGCTATAGG-3') SEQ ID NO: 4. The antisense primer was located between base 1256 and 1262 (5'-TTCTCGACTTAATGATGATGATGATGAGATGTTCGGAAGCACACACAGGGTC GGAGGCAGT-3') SEQ ID NO: 5. A SEQUENCE OF Xho I restriction site and a sequence coding for six histidine residues were introduced into 5' end of this primer for the purpose of the purifying the recombinant protein as well as for subcloning. RT-PCR of the mRNA samples from human ovaries revealed a single band of approximately 1,300 bases. This PCR product was purified and inserted into a mammalian cell expression vector, pcDNA 3.1 (Invitrogen, Carlsbad, CA). The positive clone was sequenced and found to be identical to those of the published hZP3 (Chamberlin and Dean, 1990).

Pages 43 bridging 44, last paragraph (lines 31-24), delete the existing paragraph and insert the following new paragraph:

Isolation of human ovarian mRNA and construction of cDNA for human ZP3- Total RNA was isolated from the human ovary by using the guanidinium thiocyanate method. A pair of primers was designed based on the published sequence of hZP3 cDNA with additional restriction enzyme sites and a histidine tail (12). The sense primer is located between bases 1 to 22 with Bam HI site in the 5' end (5'-TAGGATCCATGGAGCTGAGCTATAGGC-3') SEQ ID NO: 6. The antisense primer is located between base 1256 and 1262 (5'-TTCTCGAGTTAATGATGATGATGATGATGTTCGGAAGCAGACACAGGGTGG GAGGCAGT-3') SEQ ID NO: 7. A sequence of Xho I restriction site and a sequence coding for six histidine residues were introduced into 5' end of this primer for the purpose of the purifying the recombinant protein as well as for subcloning. Reverse transcription-polymerase chain reaction (RT-PCR) of the mRNA samples from the human ovary revealed a single band of approximately 1,278 bp. This PCR product was further characterized by restriction mapping, Southern blotting and sequencing analysis demonstrating identical composition to be published human ZP3(16). The PCR product was inserted into a mammalian cell expression vector, pcDNA 3.1 (Invitrogen, Carlsbad, CA). An in vitro transcription and translation system (Reticulocyte Lysate System; Promega, Madison, WI) was used to determine the molecular weight of the (non-glycosylated) protein core of the recombinant ZP3.

In the Abstract

Page 68, (lines 11-31) delete the existing Abstract and replace with the following:

The present invention provides a method to determine sperm activity comprising the steps of: (a) contacting an appropriate concentration of human zona pellucida protein 3 with an appropriate amount of sperm under conditions permitting the